

2016

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Perspectives in Dermatopathology

Histopathologic diagnosis of multifactorial alopecia

Establishing a definitive diagnosis for any form of alopecia can be challenging. Adding to the diagnostic complexity is the fact that many patients have more than one form of alopecia contributing to their hair loss. We conducted a review of 1360 consecutive scalp biopsy specimens submitted for the evaluation of scalp hair loss over a 16-month period, demonstrating that 12.5% of cases had a combination of diagnoses (multifactorial alopecia) accounting for their hair loss. An approach to the histopathologic diagnosis of multifactorial alopecia, particularly multiple forms of alopecia found in a single biopsy, is here presented.

Keywords: alopecia, dermatology, dermatopathology, hair loss, multifactorial alopecia

Wohlmann WE, Sperling L. Histopathologic diagnosis of multifactorial alopecia.

J Cutan Pathol 2016; 43: 483–491. Published 2016. This article is a U.S. Government work and is in the public domain in the USA.

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This work was supported in part by a grant from the North American Hair Research Society.

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Accepted for publication January 24, 2016

The diagnosis of alopecia can be challenging, necessitating clinicopathologic correlation and careful attention to detail by both the clinician and pathologist. Furthermore, patients can have multiple co-existing forms of alopecia accounting for their hair loss. Rendering a precise diagnosis in such cases can be difficult and complex, but can certainly be accomplished. The pathologist should be able to recognize the salient features of two or more distinct entities within the same specimen. The medical literature is largely silent about the concept of multifactorial alopecia; our attempts to find references about multifactorial alopecia were met without success, although a recent comprehensive text on hair pathology alludes to the problem.¹

Many forms of alopecia are common in the general population. Therefore it would not be unexpected for two forms to occur together in the same patient, resulting in multifactorial alopecia. The diagnosis of multifactorial alopecia is relatively straightforward when two

biopsy specimens are taken from anatomically distinct areas of the scalp (e.g. vertex and temple) and demonstrate different pathologic processes [e.g. androgenetic alopecia (AGA) and traction alopecia]. A more complex and challenging situation occurs when the changes of two separate diagnoses appear in a *single* specimen. Therefore, the focus of this article will be to present an approach to the diagnosis of multifactorial alopecia in a single biopsy specimen.

Materials and methods

We reviewed 1360 consecutive alopecia specimens submitted to a private dermatopathology practice specializing in hair loss over a 16-month period. Specimens were transversely sectioned at multiple levels from the fat up to the epidermis. Our laboratory accomplishes this by putting three separate levels on a single slide, with six to eight slides required to provide all levels from fat to epidermis. A total of 95% of the 1360 reviewed specimens were 4 mm punch biopsies, and all

cases diagnosed as multifactorial alopecia were 4 mm punch biopsies.

Results

A total of 170 individual specimens showing multifactorial alopecia (all 4 mm punch biopsies) were found; thus 12.5% of the alopecia samples demonstrated multifactorial alopecia in a single plug of tissue. The most common combination of diagnoses was AGA with central centrifugal cicatricial alopecia (CCCA), accounting for 27.1% of all cases of multifactorial alopecia. The next most commonly diagnosed combination in a single biopsy was CCCA with end-stage traction alopecia, representing 18.2% of multifactorial alopecia cases. Androgenetic alopecia with end-stage scarring (or cicatricial) alopecia tallied 17.1%, with AGA with telogen effluvium accounting for 7.6%. Additional diagnostic combinations included AGA paired with one of the following: lichen planopilaris (LPP), alopecia areata, telogen effluvium, frontal fibrosing alopecia, or fibrosing alopecia in a pattern distribution (FAPD). Six instances of AGA combined with features of CCCA and end-stage traction alopecia were found, necessitating the diagnosis of three types of alopecia within the same biopsy specimen. The complete results of our review appear in tabular form in Table 2.

In addition to multifactorial alopecia, we found 34 specimens (2.5%) demonstrating features of hair loss as well as displaying a 'non-alopecia' diagnosis such as seborrheic dermatitis. Common examples include AGA and seborrheic dermatitis, or CCCA and tinea capitis; however, numerous other combinations are possible. Out of the 34 cases, the 'non-alopecia' diagnoses found in order of decreasing frequency were: seborrheic dermatitis (18/34; 52.9%), rosacea (6/34; 17.6%), folliculitis (6/34; 17.6%), lichen simplex chronicus (3/34; 8.8%) and tinea capitis (1/34; 2.9%).

Discussion

Evaluating the specimen

Simply knowing the biopsy site can be helpful in arriving at a diagnosis of alopecia. Examples of alopecia diagnoses typically associated with certain scalp locations are listed in Table 1. As specific areas on the scalp tend to be prone to particular diseases, biopsies from those areas are more likely to show specific combinations of multifactorial alopecia (Fig. 1). For example, a biopsy from the temporal scalp might show AGA

Table 1. Common alopecia diagnoses by scalp zone

Scalp zone	Common causes of alopecia
Frontal	FFA, traction, AGA
Temporal	AGA, traction, FFA, TTA
Parietal	Traction (lower parietal/marginal)
Crown	AGA, CCCA, FAPD, dissecting cellulitis
Vertex	CCCA, AGA, dissecting cellulitis, ACC
Occipital	Traction, AKN, pressure-induced alopecia, dissecting cellulitis
Any site	AA, TE, DLE, trichotillomania (crown favored)

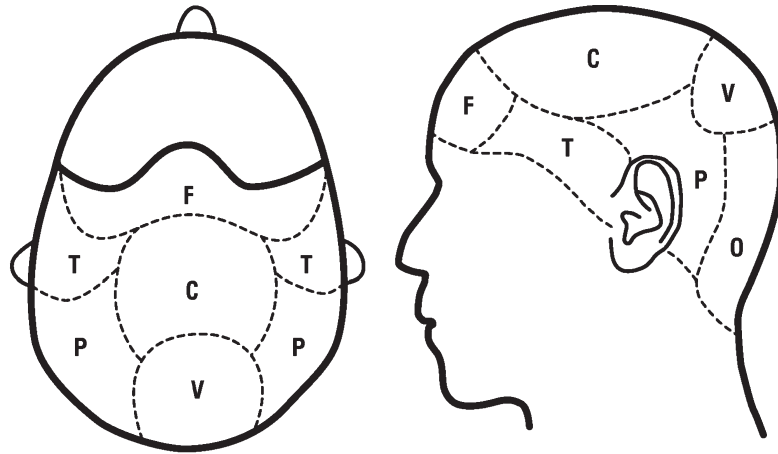
AGA, Androgenetic alopecia; FFA, frontal fibrosing alopecia; TTA, temporal triangular alopecia; AA, alopecia areata; CCCA, central centrifugal cicatricial alopecia; TE, telogen effluvium; FAPD, fibrosing alopecia in a pattern distribution; ACC, aplasia cutis congenita; AKN, acne keloidalis nuchae; DLE, discoid lupus erythematosus.

in combination with end-stage traction alopecia, frontal fibrosing alopecia, or temporal triangular alopecia; any combination of these might occur within a biopsy from this site.

The diagnosis is often dependent on finding different diagnostic features in different follicular units within the same specimen. In fact, the key to making a diagnosis of multifactorial alopecia is recognition that not all follicular units within the specimen will show features of both diseases simultaneously. For example, some follicular units may show follicular scars with residual inflammation and naked hair shafts (end-stage cicatricial alopecia); other units may show miniaturized hairs with peribulbar inflammation (alopecia areata). Thus the findings in some units are totally discordant with the findings in other units, necessitating a diagnosis of multifactorial alopecia. In order to make a confident diagnosis of multifactorial alopecia within a single biopsy, certain criteria should be met. These are as follows:

1. The biopsy is adequate for proper interpretation.
2. The histologic findings seen in the biopsy specimen cannot be explained with a single diagnosis.
3. The clinical history (if supplied by the clinician) is consistent with the proposed diagnoses.
4. In some cases, it may be necessary to have a biopsy of normal-appearing scalp (a 'control' biopsy) in order to render a definitive diagnosis of multifactorial alopecia within the alopecic zone.

The adequate specimen. In general, this will almost always require a punch biopsy at least



Zones of the scalp. Abbreviations: F – frontal; T – temporal; P – parietal; C – crown; V – vertex; O – occipital

Fig. 1. Modified from Sperling LC, Cowper SE, Knopp ES. *An atlas of hair pathology with clinical correlations*, 2nd ed. London: Informa Healthcare, 2012, page 210, with permission.

Table 2. Multifactorial alopecia diagnoses

Diagnosis	Number of cases	% of multifactorial alopecia cases	% of total cases
AGA + CCCA	46	27.1	3.4
CCCA + ESTA	31	18.2	2.3
AGA + ESTA	29	17.1	2.1
AGA + TE	13	7.6	1
ESTA + ESCA	10	5.9	0.7
AGA + ESCA	6	3.5	0.4
AGA + FAPD	6	3.5	0.4
AGA + CCCA + ESTA	6	3.5	0.4
AGA + FFA	3	1.8	0.2
AGA + ESTA + ESCA	3	1.8	0.2
AGA + Mechanical	2	1.2	0.1
ESCA + TE	2	1.2	0.1
ESCA + AA	2	1.2	0.1
ESTA + LPP	2	1.2	0.1
AKN + Mechanical	2	1.2	0.1
AGA + AA	1	0.6	0.07
AGA + LPP	1	0.6	0.07
AA + Trichotillomania	1	0.6	0.07
AA + ESTA	1	0.6	0.07
ESTA + FFA	1	0.6	0.07
ESTA + Mechanical	1	0.6	0.07
CCCA + Mechanical	1	0.6	0.07
Total multifactorial diagnoses	170	N/A	12.5
Single alopecia diagnosis	1157	N/A	85.1
Alopecia + non-allopecia diagnosis (e.g. seborrheic dermatitis)	34	N/A	2.5

MA, multifactorial alopecia; AGA, androgenetic alopecia; CCCA, central centrifugal cicatricial alopecia; ESTA, end-stage traction alopecia; TE, telogen effluvium; ESCA, end-stage cicatricial alopecia; FAPD, fibrosing alopecia in a pattern distribution; AA, alopecia areata; LPP, lichen planopilaris; AKN, acne keloidalis nuchae; FFA, frontal fibrosing alopecia.

4 mm in diameter, sectioned horizontally at multiple levels. Multiple levels are required because diagnostic features of one form of alopecia may be prominent at one level, while a second form of alopecia may only be evident at a different level. Furthermore, it is often the case that the combined histologic features seen at different levels are required for a confident diagnosis of even a single form of alopecia. It is unlikely that a confident diagnosis of multifactorial alopecia could ever be established using vertical sections. In addition, biopsies smaller than 4 mm sample so few follicular units, the likelihood of finding definitive pathologic changes is greatly reduced. Although a 4 mm biopsy contains 12.6 mm² of surface area, a 3 mm sample has a surface area of only 7.07 mm², reducing the sample area available for evaluation by nearly one half.

The histologic findings seen in the biopsy specimen cannot be explained with a single diagnosis. This is the central feature of multifactorial alopecia, and it requires familiarity with the diagnostic criteria for the various forms of alopecia. A discussion of these criteria is beyond the scope of this article, but can be readily found elsewhere.^{1–4} The specific criteria utilized in this study are found in Ref.¹ and so we will not list them here, but as an example, the criteria for AGA¹ are as follows.

1. Normal total number of follicles [about 35 (Caucasian) or 20 (African-American) per 4 mm punch biopsy] when counted at superficial dermis.
2. Reduced number of hairs (mixture of terminal and indeterminate) when counted at dermal/fat junction.

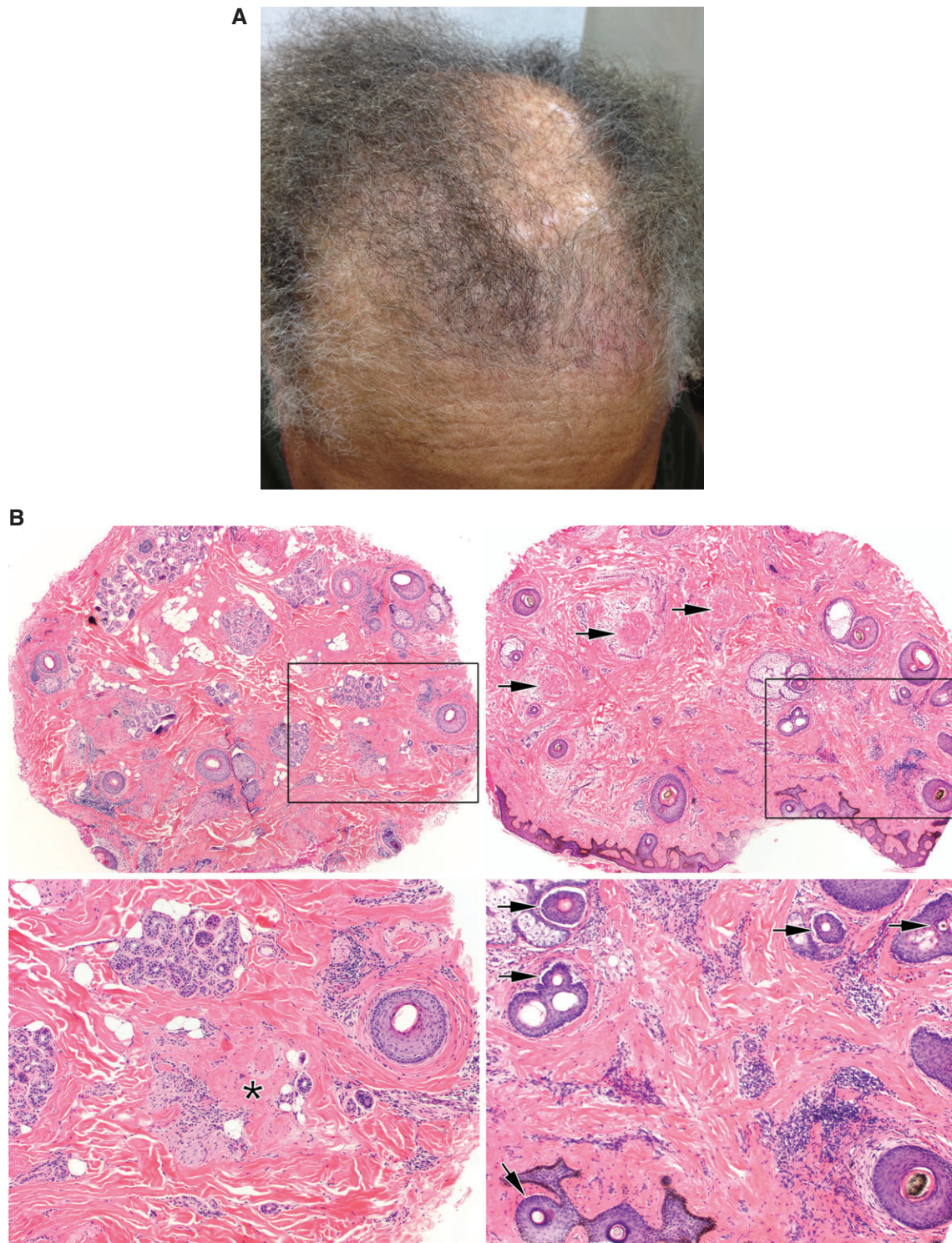


Fig. 2. Androgenetic alopecia (AGA) and central centrifugal cicatricial alopecia (CCCA). A) Clinical image demonstrating the frontoparietal thinning seen in AGA combined with the cicatricial alopecia of the crown typical of CCCA. B) Upper left panel: section through the level of the suprabulbar zone. A marked decrease in the total number of hairs is seen. The zone indicated by the box is magnified in the lower left panel. Lower left: a terminal hair shows premature desquamation of the inner root sheath. A nearby follicular scar is indicated by the asterisk. Upper right: section through the level of the infundibulum. Arrows indicate several follicular scars. There are at least 10 miniaturized hairs at this level, indicating an abnormal component of miniaturization. The zone indicated by the box is magnified in the lower right panel. Lower right: numerous miniaturized hairs are seen (arrows). A terminal hair showing concentric lamellar fibroplasia and perifollicular inflammation is seen at the lower right corner. Original magnification $\times 40$ (upper left), $\times 100$ (lower left), $\times 40$ (upper right), $\times 100$ (lower right).

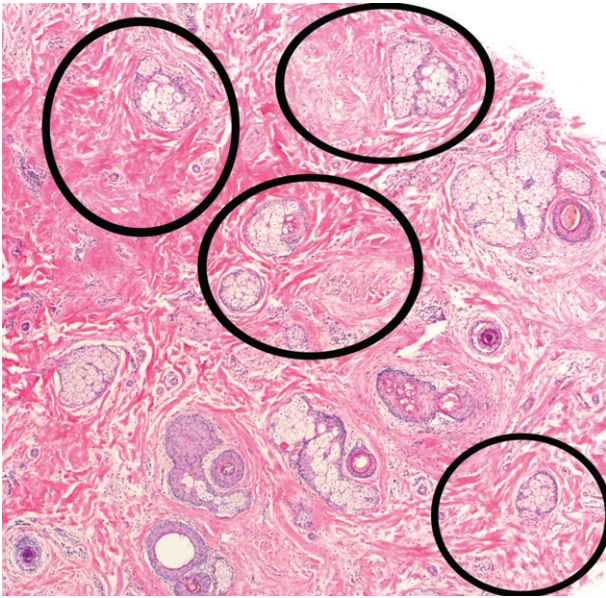


Fig. 3. Androgenetic alopecia (AGA) and end-stage traction alopecia. A portion of the section taken at the level of the upper isthmus is shown. The follicular units enclosed by circles show intact sebaceous glands but no hairs; this finding would not be present in AGA. Overall, however, the total number of miniaturized hairs is increased, indicating a component of AGA. Original magnification $\times 100$.

3. Miniaturization of hairs: a terminal:vellus ratio of 2:1 or less is typical of AGA.
4. Presence of fibrous 'streamers' below miniaturized hairs.
5. Slightly increased telogen count when compared with 'unaffected' scalp (values of 15–20% are typical).
6. Sebaceous lobules preserved but may be reduced in size.
7. No significant inflammation.

To arrive at a diagnosis of multifactorial alopecia, it is necessary to assess the total number and types of follicles and the individual follicular units (Table 2). Numerical data often plays an important role in the diagnosis of multifactorial alopecia. The total number of hairs, size of hairs and follicular phase (anagen *vs.* catagen/telogen) may point to a specific diagnosis. For example, normal or slightly decreased terminal follicle density and increased number of stela along with miniaturization of follicles (and minimal inflammation) points to a diagnosis of AGA. A normal follicular density with 20–30% of all terminal follicles in catagen/telogen and no miniaturization indicates chronic telogen effluvium (with early traction alopecia ruled out clinically). Therefore, a biopsy demonstrating normal follicular unit architecture,

miniaturization of terminal follicle diameter and 20–30% of follicles in catagen/telogen suggests a multifactorial etiology caused by both AGA and chronic telogen effluvium.

The clinical history (if supplied by the clinician) is consistent with the proposed diagnoses. For example, a diagnosis of AGA and alopecia areata would not 'fit' if the patient were a 4-year-old child, and this combination of diagnoses could not be confidently made in such a clinical setting.

Our examples represent multifactorial alopecia within a single specimen. *However, in some cases, it may be necessary to have a biopsy of normal-appearing scalp to confidently render a definitive diagnosis of multifactorial alopecia within a single specimen from the 'involved' area.* For example, a patient might show AGA and telogen effluvium in a specimen from the crown. However, as AGA typically has an elevated telogen count, a second biopsy from an area unaffected by AGA (such as the mid occiput), also revealing an increase in telogen hair follicles, would be helpful to confirm that the 'involved area' contains telogen follicles due to two processes – telogen effluvium and AGA. If a confident diagnosis requires an additional biopsy from normal appearing scalp, this can be mentioned in a comment in the biopsy report.

We will now present some examples of common forms of multifactorial alopecia (occurring in the same specimen). We will list those features supportive of each individual diagnosis, using the figures to demonstrate these features. In the examples given, we have been supplied with an adequate specimen and clinical information and/or clinical images that are consistent with the selected diagnoses. Therefore, we can focus on the histologic features.

Combination 1: AGA and CCCA (see Fig. 2)

Features supportive of AGA:

1. Follicular miniaturization (terminal : vellus ratio less than 2 : 1 with increased total number of miniaturized hairs).
2. Fibrous streamers (stela) beneath miniaturized hairs.
3. Slight increase in number of follicles in catagen/telogen may be present.
4. Solar elastosis (a feature of well-established cases) in fair skinned patients.

Features consistent with CCCA:

1. Premature desquamation of the inner root sheath.

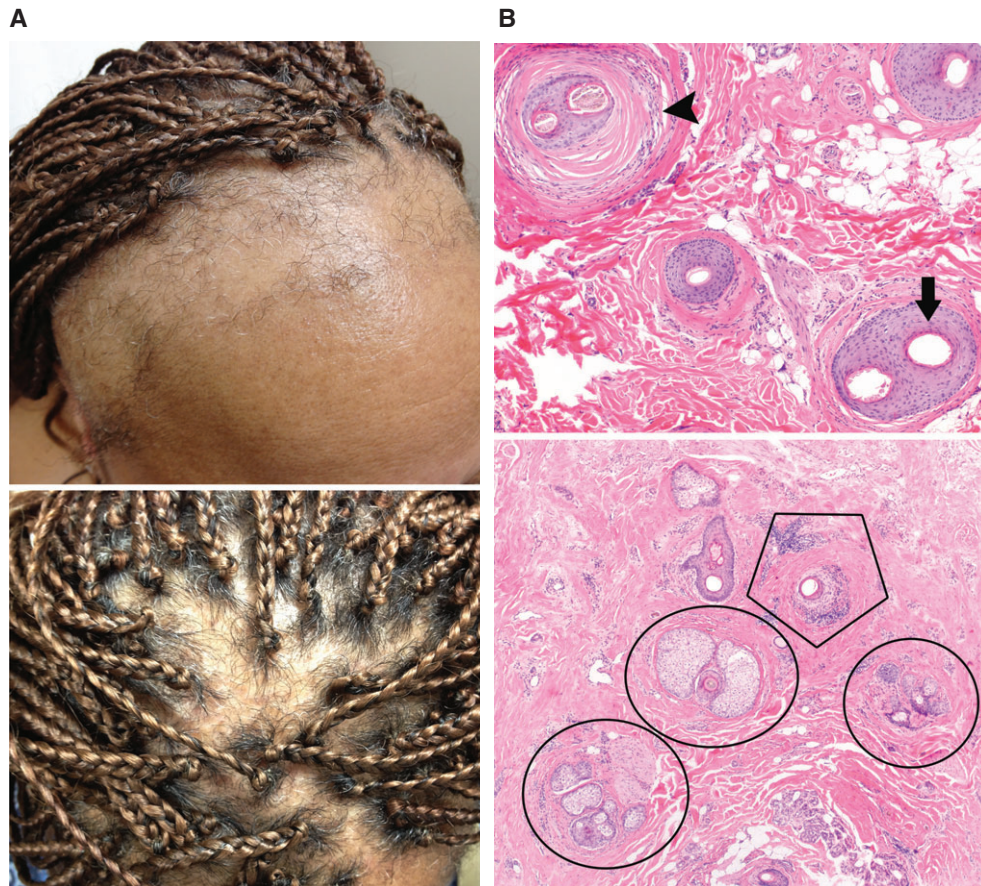


Fig. 4. A) End-stage traction alopecia and central centrifugal cicatricial alopecia (CCCA) clinical images demonstrating scarring alopecia of the fronto-temporal scalp in a band-like pattern typical of end-stage scarring alopecia (upper panel). Scarring alopecia of the crown suggestive of CCCA (lower panel). B) Top panel: a portion of the section taken at the level of the supra bulbar zone, showing typical features of CCCA, namely premature desquamation of the inner root sheath (arrow) and eccentric epithelial atrophy of epithelium with perifollicular chronic inflammation and lamellar fibroplasia (arrowhead). Bottom panel: section at the level of the upper isthmus; circled follicular units show intact sebaceous glands but only a single follicle, but one follicular unit shows loss of the sebaceous gland and typical changes of CCCA (demarcated by a pentagon). Original magnification $\times 100$ (top panel) and $\times 40$ (bottom panel).

2. Concentric lamellar fibroplasia of affected follicles.
3. Eccentric epithelial atrophy.
4. Presence of follicular scars (columns of connective tissue at the site of former follicles).

Combination 2: AGA and end-stage traction alopecia (see Fig. 3)

Features supportive of AGA:

1. Follicular miniaturization.
2. Fibrous streamers (stelae) beneath miniaturized hairs.
3. Slight increase in number of follicles in catagen/telogen.
4. Sebaceous lobules with a single miniaturized follicle.

Features suggestive of end-stage traction alopecia:

1. Marked decrease in the total number of hairs, especially terminal hairs, which are replaced by connective tissue.
2. Sebaceous lobules without associated terminal hair or even without any follicles.
3. No significant inflammation.

Combination 3: End-stage traction alopecia and CCCA (see Fig. 4)

Features consistent with end-stage traction alopecia:

1. Marked decrease in the total number of hairs, especially terminal hairs, which are replaced by connective tissue.
2. Sebaceous lobules intact.

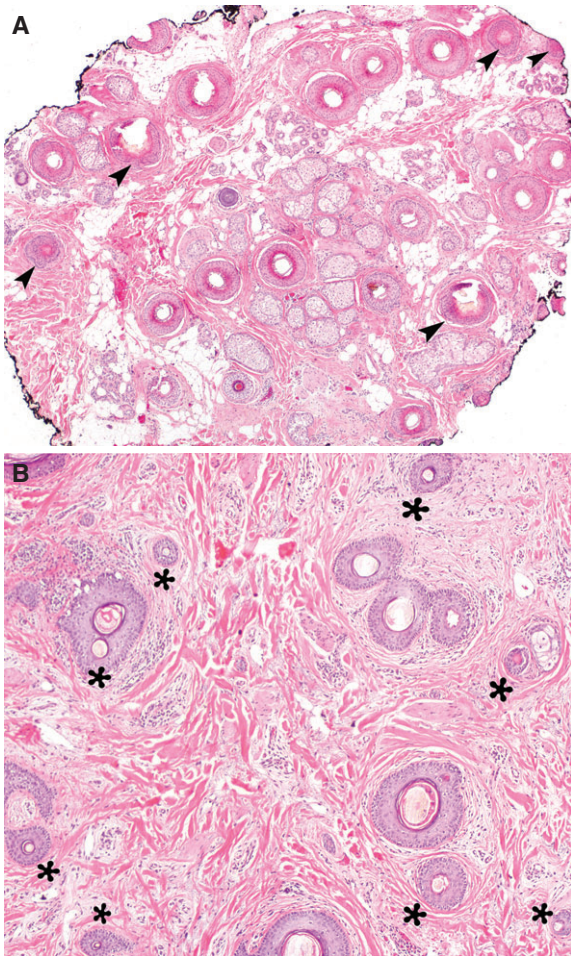


Fig. 5. Androgenetic alopecia and telogen effluvium. A) Section at the level of the suprabulbar zone showing an increased number of terminal telogen hairs (arrowheads). Original magnification $\times 40$. B) Section at the level of the infundibulum showing a markedly increased number and percentage of miniaturized hairs (asterisks). Original magnification $\times 100$.

3. No significant inflammation.

Features supportive of CCCA:

1. Premature desquamation of the inner root sheath.
2. Concentric lamellar fibroplasia of affected follicles.
3. Eccentric epithelial atrophy.
4. Perifollicular inflammation.

Combination 4: AGA and chronic telogen effluvium (this combination requires a biopsy from normal-appearing scalp to render a definitive diagnosis) (see Fig. 5)

Features supportive of AGA:

1. Follicular miniaturization.

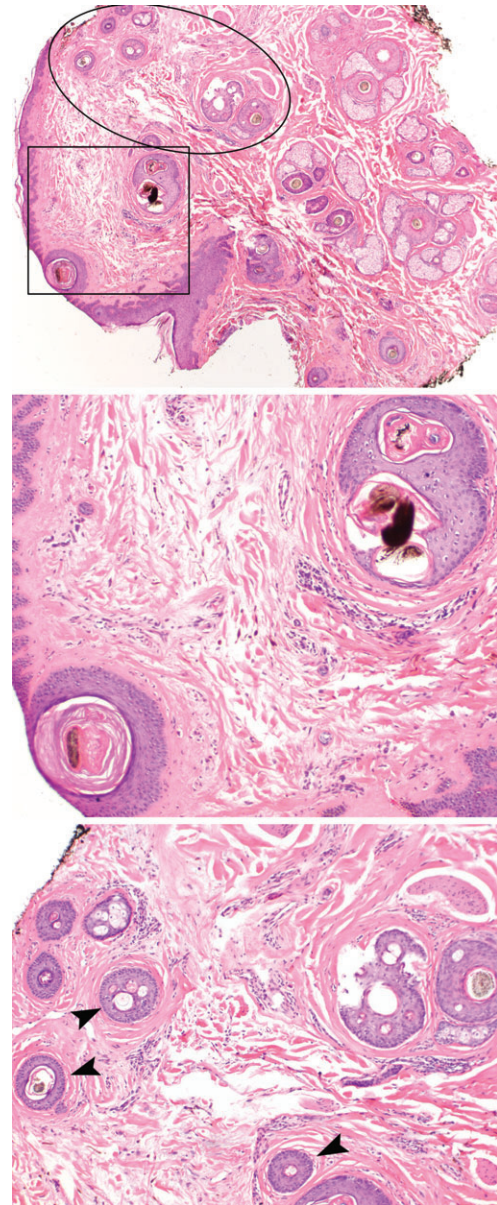


Fig. 6. Androgenetic alopecia and trichotillomania. Top panel: Section at the level of the infundibulum. The total number of hairs is normal or nearly normal, but several hairs (surrounded by a box and magnified in the middle panel) show severe trichomalacia and pigment cast formation. Overall, there is a marked increase in the percentage and number of miniaturized hairs (as in the circled area, magnified in the lower panel). Lower panel: arrowheads indicate miniaturized hairs. Original magnification $\times 40$ (upper panel), $\times 100$ (middle panel) and $\times 100$ (lower panel).

2. Fibrous streamers (stelae) beneath miniaturized hairs.

Features consistent with chronic telogen effluvium:

1. Increased terminal telogen count (greater than 20% of follicles in catagen/telogen)

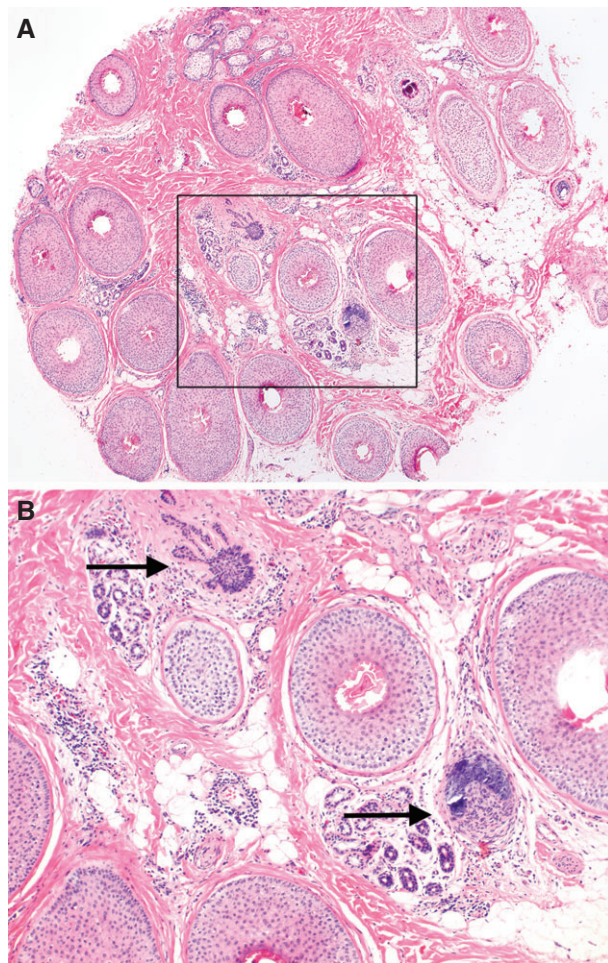


Fig. 7. Alopecia areata and trichotillomania. A) Section at the level of the suprabulbar zone showing numerous terminal anagen hairs, many of which are missing hair shafts and have collapsed inner root sheaths (distorted and incomplete follicular anatomy). The boxed area is enlarged in the lower panel. B) The bulbs of two miniaturized hairs (arrows) show peribulbar lymphocytic inflammation with a few eosinophils. Original magnification $\times 40$ (A), $\times 100$ (B).

(arrowheads) in both the involved scalp and normal appearing scalp.

Combination 5: AGA and trichotillomania (see Fig. 6)

Features supportive of AGA:

1. Follicular miniaturization.
2. Fibrous streamers (stelae) beneath miniaturized hairs.
3. Slight increase in number of follicles in catagen/telogen.
4. Solar elastosis (a feature of well-established cases).

Features suggestive of trichotillomania:

1. Incomplete or distorted follicular anatomy.

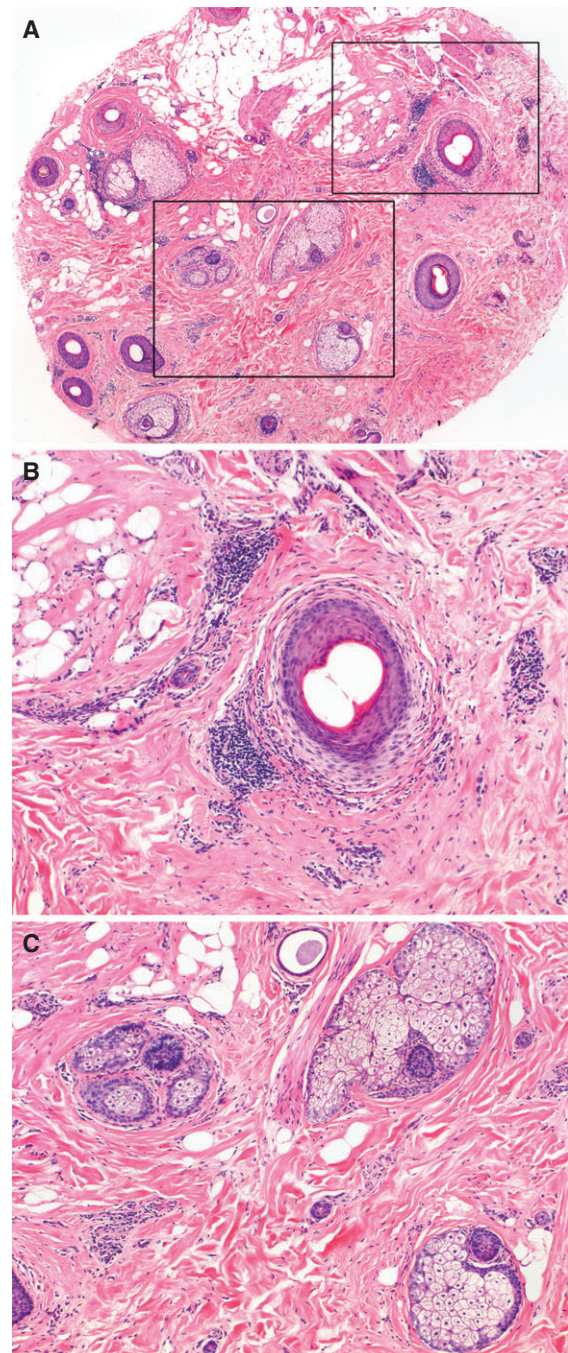


Fig. 8. Androgenetic alopecia (AGA) and central centrifugal cicatricial alopecia (CCCA) and traction alopecia. A) Low-power view of a biopsy from a patient with combined AGA, CCCA, and traction alopecia; the upper right boxed area is enlarged in the middle panel (B) and the lower left boxed area is enlarged in the lower panel (C). B) Features typical of CCCA include premature desquamation of the inner root sheath, concentric lamellar fibroplasia, eccentric epithelial atrophy and perifollicular lymphocytic inflammation. C) Features typical of traction alopecia include follicular units with intact sebaceous glands but only a single vellus hair (no terminal hairs). The total number of vellus hairs in the entire biopsy (when counted at a higher level) was markedly increased, establishing a diagnosis of AGA. Original magnification $\times 40$ (A) and $\times 100$ (B and C).

2. Twisted, linear and 'button'-like pigment casts.⁵
3. Trichomalacia can also be seen in alopecia areata.

Combination 6: Alopecia areata and trichotillomania (see Fig. 7)

Features suggestive of alopecia areata:

1. Peribulbar, mononuclear cell infiltrate with few eosinophils.
2. Increased number of terminal catagen and telogen hairs.
3. Increased number of miniaturized, aberrant hairs (nanogen hairs).
4. Pigment casts in the hair canals of vellus hairs.⁵

Features suggestive of trichotillomania:

1. Incomplete or distorted follicular anatomy
2. Twisted, linear and 'button'-like pigment casts
3. Trichomalacia can also be seen in alopecia areata.

Combination 7: AGA and CCCA and traction alopecia (see Fig. 8)

Features supportive of AGA:

1. Follicular miniaturization.
2. Fibrous streamers (stelaе) beneath miniaturized hairs.
3. Slight increase in number of follicles in catagen/telogen.
4. Solar elastosis (a feature of well-established cases).

Features suggestive of CCCA:

1. Premature desquamation of the inner root sheath.

2. Concentric lamellar fibroplasia of affected follicles.
3. Eccentric epithelial atrophy.
4. Perifollicular inflammation.

Features consistent with traction alopecia:

1. Marked decrease in the total number of hairs, especially terminal hairs, which are replaced by connective tissue.
2. Sebaceous lobules without associated terminal hair or even without any follicles.
3. No significant inflammation.

Special situation

In order to render a second diagnosis of early AGA, it may be necessary to have a sample of normal scalp for comparison.

The examples given above are the most common combinations, or show distinctive features that help to demonstrate the concept of multifactorial alopecia. Of course, many other combinations are possible. Ultimately, an adequate biopsy specimen, good clinical correlation, and the familiarity with the criteria for the diagnosis of the various forms of alopecia will be required to establish a confident diagnosis of multifactorial alopecia. In summation, we wish to introduce the concept of multifactorial alopecia, and provide a starting point for pathologists to develop expertise in the diagnosis of multifactorial alopecia. Pathologists and dermatopathologists can expect to encounter examples of multifactorial alopecia with some frequency (12.5% of all alopecia specimens in our review of cases), and so should (and can) become comfortable making the definitive diagnosis of multifactorial alopecia.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Lisa Litzenberger in the preparation of the photomicrographs and Lynda Douglas in the creation of Fig. 1.

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